IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of:

Neil H. Bander

Serial No.: 08/838,682

Filed: April 9, 1997

For: TREATMENT AND DIAGNOSIS OF PROSTATE CANCER

Group Art Unit: 1642

Examiner: Yvonne Eyler

DECLARATION OF NEIL H. BANDER UNDER 37 CFR § 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, NEIL H. BANDER, pursuant to 37 C.F.R. § 1.132, declare:

1. I hold a B.A. degree in Biology from Johns Hopkins University and a M.D. degree from the University of Connecticut Medical School.

2. I am a Professor of Urology, Weill Medical College of Cornell University where I am also the Bernard and Josephine Chaus Chair in Urologic Oncology and Surgical Director of Urologic Oncology.

3. I am an Attending Surgeon, Department of Urology, New York Presbyterian Hospital-Cornell University Medical Center.

CERTIFICATE OF MAILING
(37 C.F.R. §1.10)

I hereby certify that this paper (along with any referred to as being attached or enclosed) is being deposited with the United States Postal Service on the date shown below with sufficient postage as 'Express Mail Post Office To Addressee' in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

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4. I am also an Assistant Member at Memorial Sloan-Kettering Cancer Center, a Clinical Assistant Surgeon in the Urology Service and Clinical Immunology Service at Memorial Hospital, a Research Associate at Sloan-Kettering Institute for Cancer Research, and an Assistant Member of the Ludwig Institute for Cancer Research at Memorial Sloan-Kettering Cancer Center.

5. As explained more fully in my attached *Curriculum vitae,* I have authored numerous publications in the field of Urologic Oncology. **(Exhibit A.)**

6. I am the sole inventor of the above-identified patent application.

7. I have reviewed the Office Action mailed from the United States Patent Office on April 27, 1999, regarding the above-identified patent application. In this paper, the Examiner states at page 3 that "it is unclear if a cell line which produces an antibody...can be reproducibly isolated without undue experimentation." To the contrary, the procedures for producing the antibodies of my invention are fully described in the application. In particular, the four antibodies described in the application (i.e. E99, J415, J533 and J591) were prepared as set forth in Example 3. In fact, I used this same approach more than once to generate the four different antibodies. Antibody E99 is derived from a completely different immunization/fusion experiment than the remaining antibodies denoted by the "J" prefix, i.e. J415, J533 and J591.

8. I have reviewed Murphy et. al., "Measurement of Prostate-Specific Membrane Antigen in the Serum with a New Antibody," The Prostate 28: 266-71(I 996)("Murphy") and believe the work it describes is highly distinguishable from my present invention.

9. PSMA is a highly glycosylated integral membrane protein in a living cell and as such, it has specific 3-dimensional folding characteristics which results in exposure of epitopes, or antigen binding sites, which may not be sequential as well as the masking of certain peptides

by carbohydrate residues. Murphy utilizes the known linear peptide sequence of PSMA protein to derive and synthesize specific peptides to be used for immunization. This is a key difference from the approach I used to make the present invention. In particular, Murphy's selection of peptides is artificial and arbitrary and it cannot predictably and reliably select peptides which are, in fact, immunogenic. Furthermore, the peptide approach does not take into account the fact that PSMA is a highly glycosylated molecule and some or all of the selected peptides may be masked by carbohydrate residues of the native molecule. Of at least equal importance is that a selected peptide, because it exists somewhat in isolation and out of the context of the remainder of the molecule or, for that matter, neighboring molecules, does not possess the appropriate secondary, tertiary or quartenary structure in order to predictably and reliably generate antibodies capable of recognizing the native glycoprotein in its physiological state. Conversely, my method, by using viable, unmodified LNCaP cells, presents the native PSMA glycoprotein to the immunized animal's immune system in a form whereby generated antibodies do recognize the native glycoprotein as it exists as an integral membrane protein of a living cell.

10. Murphy describes a monoclonal antibody (called 3F5.4G, referred to herein as "3F5") produced by immunization with an 8 amino acid peptide derived from the published sequence of PSMA. This peptide resides at positions 716-723 towards the carboxy-terminus (external domain) of the protein. There is no evidence that the 3F5 antibody binds to an extracellular domain of PSMA present as an integral membrane protein of a living cell. Moreover, the data presented indicate that 3F5 may not bind to the same antigen as 7E11.C5 ("7E11").

11. In Figure I (pg. 268), Murphy provides a Western Blot with 2 lanes, 1 representing 7E11 and the other representing 3F5. These lanes represent the identical LNCaP

lysate probed with the respective antibodies. The 7Ell lane demonstrates a broad band of reactivity at a level indicated in the figure to represent PSMA. The 3F5 lane demonstrates a much narrower band purported to be at the same level. No molecular weight standards are provided to allow for comparability of the lanes or comparability of the molecular weights of the identified bands. Furthermore, the putative PSMA bands in each lane have entirely different characteristics, with the 7E11 band being much broader than the 3F5 band. The figure legend itself notes quite equivocally "that 3F5.4G6 recognizes a protein of M_r 120kDa, which is similar, if not identical, to the protein recognized by 7E11.C5."

12. In Figure 3 (pg. 270), Murphy shows an immunoprecipitate-Western-Blot where the LNCaP lysate is initially immunoprecipitated with 7E11 and then probed with either 7E11 or 3F5. If 3F5 identifies PSMA, lanes 5 and 6, which are blotted with 3F5, should closely mirror lanes 1 and 2 which are probed with 7E11. However, lanes 5 and 6 show only a very weak band of reactivity and are not even at the same level as the 7E11 bands. Lanes 3 and 7, which represent LNCaP lysate immunoprecipitated with 7E11 and subsequently blotted with 7E11 (lane 3) or 3F5 (lane 7), should also provide specific comparability of the respective immunoreactivities. However, these 2 lanes blot so much background or nonspecific immunoreactivity that they are useless in identifying these 2 antibodies as binding the same protein. Lanes 4 and 8 should show only a single band representing "PSM-prime" ("PSM'"); however, lane 8 shows 2 bands, 1 of which is neither explained nor commented on by Murphy. Figure 3b represents LNCaP lysate immunoprecipitated with 3F5 and probed in a Western Blot with antibody 7E11. Again, the 2 lanes should show identical immunoreactivities but demonstrate different patterns, further suggesting that these 2 antibodies do not recognize the same target antigen.

13. Figure 4 of Murphy provides Western Blot analyses of serum from a prostate cancer patient. Murphy indicates that the 7E11 antibody reacts with a band present in serum at a molecular weight close to that of PSMA. However, other investigators including Troyer et al. (references 5 and 6 in the Murphy paper) have published data indicating that the immunoreactivity of 7E11 on Western Blots of serum does not, in fact, react with PSMA. Troyer et al. demonstrated that one could not compete for the 7E11 binding of this serum band using a peptide containing amino acids 1-19 of PSMA which incorporates the previously mapped 7E11 epitope of PSMA. Others have also not been able to confirm that the legitimate PSMA molecule is present in serum. I have found the same cross-reaction of 7E11 with a non-PSMA molecule in serum described in Troyer et al. It is believed that the 7E11 molecule cross-reacts with a similar protein found in serum but which is different from that found associated with the cell membrane of prostate cancer cells and which would explain why the 7E11 epitope does not compete for this binding. Lanes 3 and 4 in Figure 4 of Murphy demonstrate that the 3F5 antibody identifies the same serum band as 7E11.

14. In conclusion, Murphy immunizes with a peptide, failing to take into consideration the heavily glycosylated nature and 3-dimensional structure of the native protein. He produces an IgM antibody. These antibodies are typically of low affinity and that appears to be the case with this particular antibody based on the intensity of the blot. The 3F5 antibody reacts with a molecule present in serum "identical" to the serum molecule identified by 7E11.C5. Most investigators believe that this molecule is different from prostate epithelial cell membrane-associated PSMA and that this is merely a molecule which cross-reacts with PSMA. Murphy provides no evidence, nor for that matter does he even claim, that his 3F5 antibody binds to LNCaP cells or, for that matter, that it binds to viable LNCaP cells. This antibody

appears to be entirely different from the 4 antibodies of my present invention, none of which react with a protein found in serum to an extent to give rise to background noise which interferes with *in vivo* imaging and all of which can be found to bind viable LNCaP cells. On this point, an antibody (such as 7E11 or 3F5) which binds a serum molecule would be different from, and less desirable than, an antibody which does not bind a serum molecule, if the purpose of that antibody were *in vivo* targeting of tumor. Antibody binding to a serum constituent would be 'decoyed' by the serum molecule and the antibody would never find its cellular target. The complexing of antibody in serum by the circulating molecule would further add to 'background', further diminishing *in vivo* diagnostic or therapeutic capability. In addition, the complexing of serum antigen to such antibodies bound to cytotoxic agents would be harmful to other organs.

15. It is incorrect that extracellular antigens are all internalized. Some (and probably most) such antigens are anchored in the cell membrane and do not internalize. Not all (nor even many) extracellular antigens can be induced to be internalized by antibody binding. At the time that the antibodies of my present invention were developed, those skilled in this area, as demonstrated by Israeli et al., Cancer Res. 53:227-230 (1993) and Heston, Urology 49[supp 13A]:104-112 (1997) (see pg. 106), believed that PSMA lacked an internalization sequence and therefore was not internalized.

16. Coleman, et. al., Fundamental Immunology (1989)("Coleman") is not applicable to my present invention. This reference describes the process of "capping" and "pinocytosis" and relates to cells of hematopoietic lineage not of epithelial origin such as prostate cancer. One cannot freely extrapolate observations on hematopoietic cells to epithelial cells. Indeed, the specific mechanism of internalization described by Coleman is entirely different from the internalization mechanism with respect to antibody binding to the extracellular domain of

PSMA. In particular, Coleman points out the requirement for bivalent or multivalent antibodies, indicating that monovalent antibodies are incapable of inducing internalization. By contrast, I have found that even monovalent antibodies like those of my present invention are internalized. Lastly, Coleman describes cells normally losing surface determinants in the internalization process with later re-expression of these determinants. I have never found loss of the surface expression of the surface determinants and, indeed, find that regardless of the presence or absence of antibody, the extracellular domain of PSMA is continuously present.

17. I have reviewed Israeli et al., Cancer Res. 53:227-230 (1993) ("Israeli") and believe the work it describes is highly distinguishable from my present invention. Indeed, Israeli teaches one away from my invention by indicating that synthetic peptides should be used to make anti-PSMA antibodies. In using synthetic amino acid sequences of PSMA as an immunogen to develop antibodies to PSMA, one cannot be certain how well exposed such a peptide is on a living cell, nor how immunogenic it is. Furthermore, this approach does not take into consideration the 3 dimensional folding of the native PSMA molecule, nor its glycosylation or other post-translational modifications and other characteristics which are of significant importance in an antibody response to a native antigen. Also, an immunogenic peptide may not be useful in the context of a heavily glycosylated molecule. Peptides, therefore, cannot effectively substitute for the natural tertiary and quarterary structure of a protein in a physiological situation. I am not aware of any antibodies that have been made using such a synthetic peptide of PSMA that can successfully bind to viable prostate cancer cells. I am however, aware of researchers who have tried, yet failed, at such an approach.

18. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that

these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

DATE: 7/19/99

NEIL H. BANDER